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# RECOMBINANT-DERIVED CHICKEN GROWTH HORMONE USED FOR RADIOIMMUNOASSAY

J. A. PROUDMAN

U. S. Department of Agriculture, ARS, Avian Physiology Laboratory
Beltsville, Maryland 20705

ABSTRACT. The use of recombinant-derived chicken growth hormone (rcGH) in an avian growth hormone (GH) radioimmunoassay (RIA) procedure is described. Antiserum to turkey GH bound $^{125}$I-labeled rcGH, and unlabeled rcGH or turkey GH displaced binding in a dose-related manner. The dose-response curves of sera and pituitary extract from chickens and turkeys were parallel to the rcGH standard curve. Sera from hypophysectomized (hypox) chickens and turkeys produced no dose-response and did not inhibit binding of labeled rcGH. Recovery of rcGH added to hypox sera was quantitative. Modification of the homologous turkey GH RIA protocol of Proudman and Wentworth (1) to use rcGH made possible either an increase in assay sensitivity or a 3-day reduction in incubation time.

Progress in the study of avian growth hormones has been rapid in recent years due, in large measure, to the development of homologous radioimmunoassays for turkey (1) and chicken (2) growth hormones. The chicken growth hormone (GH) assay has been widely used to measure GH in a large number of avian species (3). The turkey GH assay has been validated for measuring turkey, chicken, quail and duck GH (2), but distribution of the assay has been limited by the scarcity of purified turkey GH (or any other avian GH) suitable for use as a standard and for iodination. The original turkey GH preparation used in development of the assay, W130DB (4), was quickly depleted. Purification of turkey GH by Burke and Papkoff (5) provided limited quantities of a new standard (B181B). However, the recent purification of recombinant-derived chicken growth hormone (rcGH) produced by E. coli (6) has provided the potential for wide availability of a highly pure avian GH in theoretically unlimited quantities. The present report details the successful use of rcGH in an avian GH radioimmunoassay (RIA) procedure.

**Materials and Methods.** The RIA procedure used was a modification of the turkey GH RIA procedure of Proudman and Wentworth (1). The antiserum used was the turkey GH antiserum characterized previously. Iodination of rcGH was accomplished by the stoichiometric chloramine-T procedure using the modifications of Proudman and Opel (7). Assay tubes contained a maximum of 200 µl of sample or rcGH standard diluted to 500 µl with PBS-0.1% BSA. Tubes containing rcGH were prepared in duplicate at each of 10 log-increment doses ranging from 0.25 to 8 ng. The assay was started by the simultaneous addition of antibody solution (200 µ l of a 1:60,000 dilution) and $^{125}$I-labeled rcGH (100 µ l; about 30,000 CPM). After incubation at 4C for 3 days, precipitating second antibody solution (ovine anti-rabbit gamma globulin diluted 1:16; 200 µ l) was added. The assay was terminated after an overnight incubation by adding 3 ml of PBS. Precipatates were counted in an LKB Model 1270 gamma counter, and potency estimates were determined as described previously (1).

A variation of the above procedure was investigated for use when extreme sensitivity is required. In this procedure, the incubation protocol of Proudman and Wentworth (1) was used. The assay was started by addition of turkey GH antibody solution alone and



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incubated for 3 days. Labeled rcGH was then added and the incubation continued for 3 more days. The assay was then terminated after an overnight incubation with second antibody. The rcGH standard curve used with this protocol ranged from 0.1 to 4 ng. All other conditions were unchanged.

**Results.** Turkey GH antiserum, at a final dilution of 1:300,000, bound 28-30% of the labeled rcGH in the absence of unlabeled GH. Sera from each of four hypophysectomized (hypox) turkey poults and from five hypox chickens failed to inhibit binding at doses up to 200 µl The mean percent binding (±SE) was 103.1 ±0.8% of the binding observed without serum. The recovery of 0.5, 1 and 2 ng of exogenous rcGH added to each hypox serum was quantitative: Mean percent recovery (±SE) was 97.0 ± 1.9%. Turkey prolactin (8), which was not available when the antiserum was originally characterized, showed a cross-reaction of 0.3%.

Pituitary extracts and serum samples from chickens and turkeys produced dose response curves parallel to the rcGH standard curve and to the dose response curve of purified turkey GH (B181B)(Fig. 1). The immunological potency of the turkey GH was 56% of that of the rcGH. The within-assay coefficient of variation (CV), determined from duplicate potency estimates within a single assay, was 3.3%. The between-assay CV, determined by assaying the same serum sample in five separate assays, was 10.2%. Assay sensitivity, defined as the amount of hormone that was statistically different from zero by 2 SD, was 0.16 ± 0.03 ng (mean ± SE; n = 4 assays). Fifty percent inhibition of maximum binding (midrange of the assay) was obtained with 0.98 ± 0.06 ng of rcGH.

Statistical validation of the assay was accomplished using data from two separate but simultaneous assays of aliquots of chicken and turkey pituitary extracts and sera. The combined standard curves from the two assays (Fig.1) showed no significant deviation from linearity. The unknowns were tested for heterogeneity of regression and found to be homogeneous. The rcGH standard curve had a slope of -1.2257 logits per log unit of hormone, while the slope of the unknowns was -1.1706. Comparison of rcGH standard and unknowns by analysis of variance demonstrated parallelism between the standard curve and the dose-response curve of the unknowns as shown

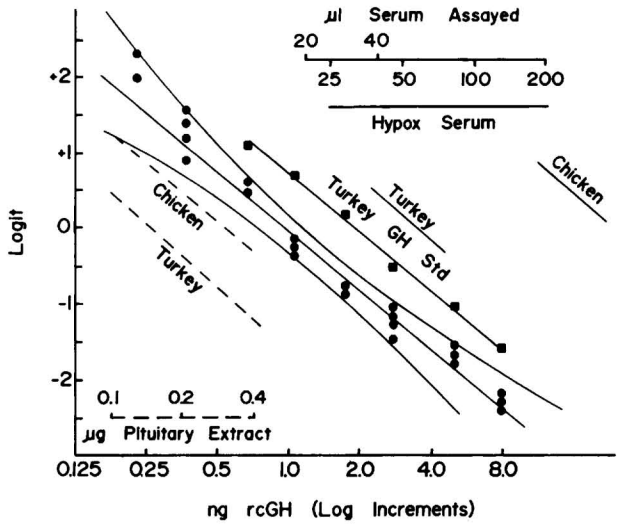


FIG. 1. Dose response curve of rcGH standard (curved lines represent 95% confidence interval) and dose response curves of pituitary extracts (---) and sera (——) from 19-wk chickens and 5-wk turkeys. The dose response curve of purified turkey GH B181B (■) is also shown. Sera from hypophysectomized chickens and turkeys showed no response.

by a nonsignificant F test for comparison of slopes and residual sums of squares (9, 10).

An increase in assay sensitivity and a reduction in the midrange of the assay was achieved by the separate addition of turkey GH antibody and labeled rcGH. This modified procedure had a sensitivity of 0.06 ng and the midrange of the assay was 0.31 ng. Potency estimates from chicken and turkey sera assayed by both procedures did not differ significantly ($P>0.05$ by paired $t$-test).

**Discussion.** Recombinant-derived chicken GH, when used with a previously characterized antiserum to turkey GH, provided an improved RIA procedure for measuring avian GH. The standard curve obtained with rcGH was linear and parallel to the dose-response curve of biological samples from chickens and turkeys. Antiserum binding of labeled rcGH in the absence of added GH was higher than that achieved with any turkey preparation used in this laboratory. The immunological potency of rcGH was nearly twice that of highly purified turkey GH. These improvements in assay binding and potency permitted modification of the homologous turkey GH RIA protocol to achieve a 3-day reduction in incubation time without any loss in sensitivity. Alternatively, the more lengthy protocol provided substantially improved sensitivity when used with the rcGH. Thus, the RIA user has the choice of a more rapid assay for use in studies of growing birds, or an extremely sensitive assay for measuring the very low GH levels of adult birds. However, perhaps the greatest advantage that may acrue through use of rcGH for RIA will be the improved comparability of results between laboratories that a widely-available standard hormone preparation can provide, and the increased use of avian GH RIA's that were previously limited by the scarcity of purified hormone.

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